

I / We claim

1. A method for preparing ready-to-use solid support for rapid ELISA, wherein the said method comprising the steps of:
  - 5 a) adding a first monoclonal antibody dissolved in coating buffer to the wells of the solid support and incubating the solid support at about 4°C for a period ranging between about 12 and 14 hours for binding the first monoclonal to the solid support;
  - b) washing the solid support of step (a), with a washing buffer to remove the unbound monoclonal antibody;
  - 10 c) adding a stabilizer solution to the wells of the solid support of step (b), incubating for a period ranging between 12 and 14 hours at about 4°C;
  - d) decanting to remove the stabilizer solution of step (c), and completely drying the wells of the solid support;
  - 15 e) adding to the wells of the solid support of step (d), an appropriate second antibody and an appropriate third antibody conjugated to an enzyme dissolved in a suitable buffer containing the blocking agent; and
  - f) freeze drying the plate of step (e), storing the plate in a sealed pack at a temperature range of about 4-8°C for use.
- 20 2. A method as claimed in claim 1, wherein the first monoclonal antibody is raised against the protein/antigen to be detected.
3. A method as claimed in claim 1, wherein in step (a) the first monoclonal antibody used is selected from a group consisting of monoclonal antibodies raised against Cry proteins and monoclonal antibodies against 5-enolpyruvylshikimate-3-phosphate synthase, wherein Cry protein is preferably selected from Cry1Ab, Cry1Ac Cry2Ab, Cry 9A, Cry 9B and Cry 9C.
- 25 4. A method as claimed in claim 1, wherein in step (a) coating buffer used is selected from a group consisting of carbonate buffer and phosphate buffer, having pH in the range of 9.0-9.8.
- 30 5. A method as claimed in claim 1, wherein in step (b) the washing buffer used is phosphate buffer saline having a pH in the range of 6.8-7.2.

6. A method as claimed in claim 1, wherein in step (c) the stabilizer used is selected from a group consisting of a Phosphate Buffered Saline, Fish Gelatin and Glycerol mixture and a Tris-buffer, Fish Gelatin and Glycerol mixture.
7. A method as claimed in claim 1, wherein in step (d) the drying method used is  
5 either freeze drying or lyophilisation
8. A method as claimed in claim 1, wherein in step (e) the blocking agent used is selected from the group consisting of ovalbumin, bovine serum albumin, bovine nonfat milk powder, casein, fish gelatin, porcine gelatin and lambda-carrageenan.
9. A method as claimed in claim 1, wherein the solid support used is selected from  
10 the group consisting of ELISA plate and microwell plate.
10. A method as claimed in claim 1, wherein the material for the solid support used is either polystyrene or polypropylene.
11. A method as claimed in claim 9, wherein the solid support is made of polystyrene.
- 15 12. A method as claimed in claim 1, wherein the second antibody used is polyclonal antibody IgG raised against protein/antigen to be detected.
13. A method as claimed in claim 1, wherein in step (e), the second antibody is a polyclonal antibody IgG raised against corresponding Cry protein or IgG raised against 5-enolpyruvylshikimate-3-phosphate synthase.
- 20 14. A method as claimed in claim 1, wherein in step (e), the third antibody is selected from the group consisting of polyclonal whole IgG conjugated to an enzyme, wherein whole IgG may be obtained from class Mammalia or class Aves.
15. A method as claimed in claim 14, wherein the enzyme used is selected from a group consisting of alkaline phosphatase and horseradish peroxidase.
- 25 16. A rapid method for performing ELISA using ready-to-use solid support obtained in claim 1, wherein said method comprises the following steps:
  - a) reconstituting the ready to use plates by adding appropriate amount of distilled water;
  - b) adding to the plate of step (a), samples containing antigen/protein to  
30 be tested dissolved in a suitable buffer, incubating the plate at about 35 to 40°C for about one hour for forming an immunocomplex with the bound first antibody;

- c) washing the plate of step (b) with a suitable washing buffer to remove the unbound antigen;
  - d) adding to the plate of step (c), a buffer containing chemical substrate and incubating for about 30 minutes in dark at room temperature; and
  - 5 e) detecting for the presence of the antigen by measuring absorbance in step (d) at a suitable wavelength
17. A method as claimed in claim 16, wherein in step (e) wavelength suitable for measuring the absorbance is between 400-700 nm.
18. A method as claimed in claim 16, wherein in step (d) the chemical substrate is  
10 selected from the group consisting of para-nitrophenol phosphate, Nitro Blue Tetrazolium/5-Bromo-4-Chloro-3-Indolyl Phosphate, 2,2'-Azino-bis (3-Ethylbenz-thiazoline-6-Sulfonic Acid), o-Phenylenediamine, 3,3'-5,5'-Tetramethylbenzidine, o-Dianisidine and 5-Aminosalicylic Acid.
19. A rapid ELISA kit comprising of:
- 15 a) a ready-to-use solid support for detection of protein or antigen to be tested,
  - b) wash buffers,
  - c) chemical substrate,
  - d) substrate buffer,
  - 20 e) stop solution,
  - f) positive and negative control samples, and
  - g) an instruction manual
- 19 A ready-to-use solid support for detection of protein or antigen
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